EXHIBIT 16

(7)

[redacted]

Consecutive digestion of 4-1BBtag/BglII ± HindIII

BglII digestion ▼19 ml

water	11 ml
React I	2 ml
HindIII	2 ml
	40 ml at 37°C

(purify by elutip method) 05:00~06

digestion of 4-1BB PCR products

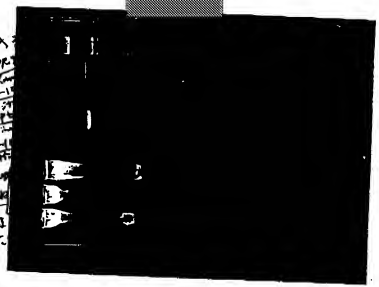
4-1BB (#3, #4)	16 ml	4-1BB (5', 3')	16 ml
React 3	2 ml	React 3	2 ml
spermidine	1 ml	spermidine	1 ml
EcoRI	1 ml	BglII	1 ml
	20 ml		20 ml

~~phenol/chloroform ext. (and ethanol ppt)~~ at 37°C 05:00~06

add React 3 (	2 ml	React 4)	2 ml
water	16 ml	water	16 ml
StuI	2 ml	HindIII	2 ml
	40 ml		40 ml

05:45

(7)



Ligation

	Exp.	Control (self)
pPR998/XmnI-RI (25 ng/μl)	4.0 μl	4.0 μl
Q-IBQ CPCK/StuI-RI (12 ng/μl)	0.5 μl	—
5X buffer	2.0 μl	2.0 μl
water	3.0	3.5
Ligase	0.5 μl	0.5
	10.0 μl	10.0

Ligation

	exp.	self
APtag/HindIII..BglII (150ng/μl)	1 μl	1 μl
4-1BB(PCR)/HindIII-BglII (8ng/μl)	2 μl	—
5X buffer	2 μl	2 μl
water	4.5	6.5
ligase	0.5	0.5 μl
	10 μl	10.0 μl

mutant (self)
4.0 μl
—
2.0 μl
3.5
0.5
10.0